

*A Physiological Theory of the Calcification of the Skeleton.*

*By Prof. John A. Ryder.*

*(Read before the American Philosophical Society, November 1, 1889.)*

The well-known researches of Harting, Rainey (1858), and Ord (1879) on the interference exerted by the physical properties of colloids in modifying the form of crystalline bodies left to crystallize therein, may afford the basis for an interpretation of the processes attending the genesis of the calcareous skeleton in many living forms. Especially is this true if we keep in view the significance of the indifferent intercellular colloids normally produced at certain places in the living organism, and their comparative passivity with reference to all of the metabolic processes going on in the surrounding active or cellular tissues. The substances which, when isolated, as Collagen, Elastin, Chondrigen, Chondrin, etc., represent in a separate form the basis of the non-cellular, passive and supporting structures or skeletal elements which serve as points of attachment for the apparatus of motion, the muscular system. These materials, which are essentially of intercellular origin, represent the colloidal or fibro-laminar matrix of bone and cartilage throughout the vertebrates, in which calcareous matters are thrown down and retained, so as to give more or less firmness or rigidity; or, as a firm jelly, as in the case of cartilage, in which cells are imbedded, a certain rigidity is attained through the molecular stability and cohesion of the structures so formed, as in bars of cartilage, for example.

The one series of features which characterizes these bodies is their intercellular origin, their homogeneity and molecular stability or inactivity. They therefore stand in the most extreme contrast with respect to their physical properties when the latter are compared with the other active, living cellular tissues of the organized bodies in which they are found. While all of the living cells of the organism exhibit an active metabolism, the non-cellular supporting tissues, such as the white fibrous, yellow elastic and cartilaginous, cannot of themselves exhibit anything of the sort, but only through the intermediation of the vascular and other tissues is such metabolism possible. Cartilage is usually not traversed by vessels, and is never richly vascular, though it may give passage to a few widely scattered vessels, as happens in some of the cranial cartilages of the sturgeons. As a rule, the presence of vessels in cartilage carries the implication that they have grown into the cartilage secondarily; myeloplaxes or other amœbiform cells have eroded the cartilage in advance of the in-growing vessel. In the highest types of bone development, as met with in mammals, birds, reptiles and Amphibia, this is the way in which the cartilage is removed from the centres of long bones, after it has served its purpose as a matrix upon which the forms of the permanent skeletal elements have been moulded in the form of the firmer and more stable substance which

eventually forms the matrix of the calcified skeleton of the adult. This new matrix, after the hollowing-out process has been accomplished by the agency of the ingrowth of the blood vessels and amœbiform osteoclastic cells into the cartilage, is deposited not only within the bone but also on its outside; at first the amœbiform cells, which now begin to be included within it, as the bone substance grows in thickness, are known as osteoblasts, and are joined together by fine protoplasmic processes and to the lymph and blood spaces which have been eroded by the latter within the bone substance. In this way an elaborate metabolic cycle is established with the blood vascular system in which the fine protoplasmic threads, joining the bone cells into an almost infinitesimally fine reticulum, are the ultimate ramifications, while the system of blood and lymphatic vessels are the gross bonds through which the whole is brought into relation and continuity with the general metabolism of the body.

The ultimate ramifications of the vessels through the adult bony tissue are known as the Haversian canals and the canals of Volkmann. The bony matrix around the former is concentrically laminated, around the latter it is not. In the very young of higher animals, such as a child under a year old, the bony tissue does not exhibit the lamination around the Haversian canals such as it shows in the bones of the adult. This interesting fact is confirmed by the structure of the bones of fishes, in which there may not even be osteoblasts present within a bone at any period of the life of the animal; the bones being in reality nothing but absolutely homogeneous or laminated plates of a matrix which has calcified throughout. The matrix in this case, as in all the others, has been deposited by the action of connective-tissue cells and vessels, and both of these may be observed in the vicinity but lying external to the bone matrix. In other cases an elaborate reticular calcifying matrix is developed within cartilage without the presence of vessels. The most singular type of this is that met with in the vertebral centra of sharks, where the radiating and concentric fibres of the calcifying matrix arise between the cartilage cells which formed the primary or embryonic vertebral body. The radiating fibres, in this case, may be traced as continuations into the fibrous connective tissues investing the vertebral column. This matrix is homogeneous, highly refringent and its origin may be traced in the embryo directly to the *membrana elastica externa* of authors.

The non-vascular character of all the tissues of animals immediately involved in calcification has been thus well established. The only vessels which can be identified as actually perforating as the minutest canals either bone matrix or cartilage are the canaliculi from the lacunæ in which the bone and cartilage cells lie. The comparatively coarse capillaries of the Haversian systems are remnants of the erosive and constructive processes which took place when the bone was built up during ontogeny. They become narrower and more constricted as life advances, and the bones become more solidified. It is therefore obvious that the processes of metabolism are here normally at a very low ebb so far as they affect the

bony tissues proper. The matrix being firm and stable there is no close and continuous union with the vascular system except by way of the network of processes of the so-called osteoblasts or "bone-forming cells," which indirectly form one system with the Haversian systems of blood and lymph vessels in the higher types. In the lower types and in embryos this is not the case; the bone matrix as a plate or delicate reticulum (vertebræ of sharks) calcifies without even the presence of so-called osteoblasts embedded in its substance. The calcification of the homogeneous concentrically laminated cysts, containing parasitic *Trichinæ* in infested muscle, the cysts being the result of the defensive action of the connective tissues and vessels against the parasites, is surely proof that calcification is a physical process determined merely by the presence of an ametabolic or stable colloidal matrix, which is capable of taking up calcareous or other earthy salts, the latter losing their crystalline characteristics more or less completely, owing to the physical interference of the colloid in the processes of crystallization.

In young sharks, eight inches long, the lower jaw is instructive in this connection. Cross sections of it show that the calcareous salts have been deposited on the superficial layers of the matrix of Meckel's cartilage as irregular nodules, many of which recall some of Rainey's figures of crystalloids formed in artificially prepared and calcareously saturated colloids. These nodules in the young shark's jaw are, moreover, nearest the surface of the cartilage or nearest the vascular source of calcareous deposit. In developing bone the same holds true. It is in the middle or diaphysial part of the cartilage of a foetal long bone in which endocartilaginous calcareous deposits are first to be observed, or in the oldest part of the cartilage or that in which the colloidal matrix has had the most time in which to passively take up such materials. Later this calcareous material is again absorbed when the cartilage of the shaft is eroded and destroyed by the ingrowth of the blood-vessels, at the time the formation of the matrix of the true bone is to begin.

Summing up the whole of this evidence, we are fully warranted in drawing the following inferences:

1. That a dense homogeneous, a metabolic or passive colloidal matrix, whether found within or without a living body, will tend to abstract and precipitate within itself, in the form of nodules or granules, any calcareous salts with which it may be mixed or by which it is bathed.
2. That the density of these colloidal matrices greatly increases their avidity for calcareous matters, so as to cause them to very soon become completely saturated with the calcareous salts so as to appear homogeneous, and characterized by a mineral-like brittleness, as in the laminar bones of fishes, without included osteoblasts, or as happens in the case of the dense matrix of the true bone tissues of higher animals.
3. That the firmness and persistency of the bones of higher forms are due to the presence of their matrix of collagen-like material to and through which the calcareous materials are being continually brought by the

blood and lymph vessels of the marrow and periosteum, to be absorbed and carried to all parts of the thick bone substance by the processes of the bone cells or osteoblasts.

4. That the avidity of the absorption of calcareous matter is proportional to the density of the colloidal matrix, and that the gradually increasing brittleness of bone, as age advances, is due to such increasing density of the matrix and its still more enfeebled and less active metabolism.

5. That the feebleness of the metabolism going on in bony tissue or matrices is the main cause, together with their avidity for earthy salts in solution in the fluids of the body, of the rapid calcification of young bones as well as of the persistence of their calcified condition throughout life.

6. There are no such things as "bone cells" or "osteoblasts" in the sense of makers of bone; these cells play an absolutely intermediary rôle in bone formation, since they only give rise to the bone matrix, and form a network in the highest forms of bone by which a bond of union is established throughout its substance, serving for the transmission of calcareous salts from one part to another. The absorption and retention of calcareous matter by bones is a physical process, conditioned by the establishment and circumscription of colloidal or homogeneous stable matrices at definite points in the organism; these loci in turn are determined by the soft parts through inheritance.

7. The soft parts have determined, in the main, the shapes of the hard parts of the endoskeleton, and not *vice versa*, with the exception only of the teeth.

The earliest form of a colloidal non-cellular matrix is found in the umbrella of Medusæ, of both hydrozoan and scyphozoan types. It here confers an elasticity to the umbrella which is sufficient to effect the recoil of its margins when the animal is in motion. In other words, the systole of the umbrella is effected by the marginal radial and circular muscles, its diastole or recoil is due to the elasticity of the mass of elastic more or less colloidal matter interposed between the epiblast or ectoderm and the hypoblast or endoderm. Its function is here not only that of a supporting endoskeleton which gives the umbrella its configuration, but also effects the diastole of the umbrella without involving any extra expenditure of energy through its own metabolism, as must happen in contracting muscle.

This gelatinoid matrix is a secretion of the primary embryonic layers and plays an important rôle in giving form or configuration and a certain rigidity to many larval forms. It is therefore physiologically the oldest of all endoskeletal structures.

The next element of the endoskeleton which is of equally great significance in the elaboration of a theory of the genesis of the vertebrate endoskeleton are the axial cellular cords of the tentacles of many Hydrozoa and Scyphozoa, the oral rods and axial supports of the cirri of Branchiostoma, and the notochord of all vertebrates, ascidians and Hemichordata. Prob-

ably the vesicular connective tissue of mollusks falls into the same category.

The common character of all these types of tissue is the development of cavities in its cells filled with fluid. All, except the last, also seem to have lost the power of storing up plasma, and the plasmic cell walls become thin and the nucleus is either pushed into a parietal position and adherent to or embedded in the cell wall, as in the notochordal tissue, or the nucleus is suspended by plasmic threads running radially from the plasma which invests it to the attenuated wall of the vesicular cell.

A mass or cord of such cellular tissue also possesses certain elastic properties as a result of which it may not only give form but also save the expenditure of energy in the same way as we have already seen in the case of the gelatinous matter of the umbrella of *Medusæ*. Its elasticity probably serves to automatically straighten out the body in young fishes or those in which the notochord is persistent, and thus saves the expenditure of a great amount of energy through the metabolism of muscle. In fact it is almost absolutely certain that the notochord of embryonic vertebrates has the elastic properties which I ascribe to it, judging from the phenomena exhibited while such embryos are dying under actual observation. It is a truly cellular supporting tissue in contradistinction to the jelly-like matrix which makes up most of the sometimes slightly fibrous substance of the umbrella of *Medusæ*.

The elastic properties of notochordal and other vesicular supporting tissues do not arise as in the gelatinoid non-cellular supporting matrix from an inherent elastic property such as would be met with in a mass of rubber or a jelly prepared from gelatin, but from the qualities arising from a cord or mass of minute cellular vesicles, with rather firm walls, bound together by intercellular substances and external cuticular and intercellular membranes, investing such an aggregate of hollow cells. Such a mechanical aggregate possesses somewhat the properties of erectile tissue, the qualities of which depend upon turgescence. In the latter case it is temporary vascular turgescence under the control of the nervous system, and consequently involves the expenditure of energy through metabolism, both nervous and muscular, whereas in the former case the permanent erection of the tissue is due to the permanent turgescence of the tensely filled individual cells and calls for apparently no expenditure of energy.

Another property of such vesicular tissues is their passivity or metabolic indifference, which is obvious from simple inspection. This indifference or metabolic passivity is also exhibited *en masse*, since there is invariably a tendency to form more or less homogeneous fibrous or dense cuticular membranes around such masses or cords of cells. This is the expression in the living normal organism of the same phenomenon as the deposition of a cuticular capsule around a sliver or bullet which has penetrated and become permanently embedded in the comparatively indifferent or passive connective tissues of a higher animal, or the deposition of a cuticular laminated capsule around *Trichinae* lying between muscular

fibres, or the young trematode which has bored as a *Cercaria* into the corium of a fish and there become encapsuled.

It will be seen later that these facts have a profound significance as respects the genesis of the endoskeleton met with in many mollusks, ascidians, balanoglossus and vertebrates.

In the vertebrates the first intimations of cartilage are met with in *Branchiostoma* around the notochord, but not as definite bars. The gelatinoid matter for the matrix appears to be present around and between the notochord and the muscle plates and in the epipleural folds, but no definite loose cell aggregations have as yet wandered into it to constitute true cartilage. No true cartilage, in fact, exists in *Branchiostoma*, only the materials and possibilities of it.

In marsipobranchs the neural and hæmal arches are developed around the anterior part of the notochordal axis only, and these elements here present the character of a tissue of cartilage cells embedded in a dense fibrous matrix the origin of which is, however, to be traced to a homogeneous circumnotochordal gelatinoid matrix such as is seen investing the notochord of *Branchiostoma*.

The next step in advance is made through the chimæroids and sturgeons, in which the cartilaginous arches are developed for nearly or quite the entire length of the notochord. From this point onward the cartilage begins to preponderate around the notochord, and as we rise in the vertebrate scale the neural and hæmal arches, where they abut against the notochord, expand in all directions as flat disks, so as to form a more or less complete investment around the notochord. Eventually in birds and mammals, the cartilage precociously replaces the notochord, and it dwindles or aborts in the embryo so that by the time the latter is hatched or born, traces only of the notochord remain within and between the centra.

The axial intermuscular tissue in the extremities of higher types seems to have given rise, in the same way as above described, to the cartilaginous matrix of the limb-bones. In *Branchiostoma* traces of continuations of the gelatinoid intermuscular substance is found in the epipleural folds. In the true vertebrates cords of proliferated cells in the axis of the limb (prochondral cells of Strasser), pour out or aggregate to themselves more and more of the primitive intermuscular and intermembranous gelatinoid matrix. Definite bars of cartilage so arise, at first unsegmented, representing the whole of the future osseous merites or segments of the limb. At first not sharply circumscribed, such bars eventually become definitely and sharply outlined and imbedded in a matrix of fibrous connective tissue, which is the perichondrium or germ of the future periosteum, in case future ossification occurs.

With circumscription of the definitely formed bars of cartilage the exudation of a still more dense deposit of homogeneous matter occurs through the instrumentality of the perichondrium. This is invariably thickest at the oldest part, or at the middle, of the shaft or diaphysis of cartilage

representing a long bone. This deposit is at first thickest around the middle of the long bones for the same reason as that already assigned for the early appearance of calcareous deposits in the middle of the diaphysis, viz., the greater age of this region.

In calcified bones of Batrachia, Kastschenko has shown that these first homogeneous deposits contain no bone corpuscles, an observation which I have confirmed, while Kölliker has lately recorded similar facts regarding the bones of very young Mammalia, while my own studies in fishes show that there are forms, such as the *Lyomeri*, which reach even the adult stage without including a single osteoblast in any of their calcified bones. I may further add that embryonic membrane bones of Mammalia, and especially of fishes, at first develop and calcify in the form of absolutely homogeneous plates or in reticuli of a dense homogeneous substance allied to collagen.

Summarizing these results still further, it may be said that :

1. The indifferent intercellular colloid or gelatinoid tissues of invertebrates and vertebrates have a labor-saving as well as a supporting function.

2. The same may be said of the indifferent or ametabolic vesicular and notochordal tissues of invertebrates and vertebrates.

3. The indifferent, ametabolic or passive qualities of both these kinds of tissues apparently leads to the throwing down of homogeneous cuticular deposits upon their surfaces by the surrounding tissues, in much the same way as indifferent foreign bodies are encapsuled by colloid laminated membranes, and which may become infiltrated with calcareous matter.

4. Osseous or calcareous infiltration of gelatinoid or colloid matrices, and of homogeneous reticuli or membranes, always begins in the oldest parts of the same, in conformity with what would be expected of them on a *priori* grounds and in view of their ametabolic physical properties.

A homogeneous membrane of collagen may give rise by intercellular extension and deposit to a reticulum consisting of the same substance. This happens in the vertebræ of sharks, where such a reticulum invades in an outward direction the investing rings of cartilage forming the bodies of the vertebræ and calcifies directly without the intermediation of the invasive or irruptive processes which accompany the formation of the cancellous tissue of the bones of many vertebrates, including Teleosts, Batrachia, Reptilia, Aves, Mammalia.

The cartilaginous tube investing the notochord in sharks has probably been evolved through a condition which is permanent in *Chimara*, where the cartilage at the bases of the neural and hæmal arches has begun to irrupt through the outer *membrana elastica externa* into the here enormously thickened fibrous *membrana elastica interna*. In Teleosts the elastic sheath in many cases seems to calcify and segment directly into the vertebral rings. In other forms the *elastica externa* is the first to calcify, even long before the cartilage of the vertebral centra. The *elastica externa* is rudimentary in Aves, Reptilia and Mammalia, and can be seen

only in the embryo with fine processes sometimes extending in cross sections a little distance into the here more developed hyaline matrix of the massive cartilaginous investment.

When the irruptive processes of bone formation or neoplastic ossification occur they are followed by the deposition of dense homogeneous or fibrous matrices around the marrow cavities so formed, within the original homogeneous calcareous tube, which was formed by moulding a deposit of collagen-like substance upon a matrix of cartilage. In such new homogeneous deposits new infiltrations of calcareous matter occur within and superimposed upon the old. In such endosteal deposits and in the later periosteal deposits the homogeneity of the bone substance is interrupted only by the intercalation of the osteoblasts which are left imbedded in the homogeneous matrix as the latter is laid down, layer after layer.

If time permitted, the mechanical advantages and expedients of neoplastic bone formation, as well as the intricate series of substitutions which can be traced in the evolution of the vertebrate skeleton, might be discussed in full, but my intention has been fully realized, at present, if it has been made clear that the physical, chemical and physiological properties as well as the mechanical space relations of the parts involved, must be considered in order to arrive at a sound theory of the origin of the skeleton. It is especially to be insisted upon that the physical properties of the substances involved in calcification are to be taken into account quite as much as their morphological characters, in working out a general theory of the history of the skeleton. Morphology may serve to aid in interpreting phylogenies, but it is evident from what has preceded, that morphology alone is incapable of grappling with the question of the true causes of the genesis of the skeleton, quite as much so as the overstrained hypothesis of natural selection.

NOTE.—The only portion of the skeleton of vertebrates, the matrix of which is of exoskeletal origin, is the enamel. The dentine is endoskeletal.

The elastic fibres found by Kölliker in bone, belong to the category of substances denominated colloidal. The later fibrous character of the matrix of many if not the majority of bones of higher types, as a result of which, while living and moist they have a certain elasticity, does not any the less exclude their constituent fibres from the group of colloidal substances. In the same way the homogeneous actinotrichia of the fins of fishes and their investing matrix of calcified matter is colloidal. The precipitation of calcareous matter follows these actinotrichia to the very margin of the fin folds, where they diverge more and more like the ribs of a fan, and thus give rise to the characteristic dichotomous character of the soft rays of all malacopterygian forms of fishes.

The deposition within the middle of the notochord of an axial median cord of fibrous collagen-like substance is a further instance of the slow degeneration and metabolism which may go on within even a relatively indifferent tissue. This axial cord is an intercellular deposit, and has been



frequently mistaken by anatomists for the whole of the notochord, in the intervertebral regions of the column. It is present in *Chimara*, *Acipenser*, *Petromyzon*, *Myxine*, and traces of it are present in higher types. It may be derived from the primitive central canal of the notochord, or mark the point from whence that canal has disappeared. The partially tubular notochord of Hemichorda would countenance this view as well as the partially hollow notochord (at the caudal region) of the embryos of some birds.

The writer would here acknowledge the value and importance to him of materials kindly supplied to him from the National Museum at the instance of the obliging Director of that institution, Dr. G. Brown Goode.

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*On the Genus Nyctinomus and Description of Two New Species.*

*By Harrison Allen.*

(Read before the American Philosophical Society, October 4, 1889.)

The genus *Nyctinomus* includes twenty-one species and is of cosmopolitan distribution. Thus ten species are found in Africa and Madagascar; one in Europe with a range in the northern part of Africa; two in India; two in the Malay Archipelago; one each in Polynesia and Australia, and four in America. It is interesting to contrast this wide range with that of the other two genera of the group in which *Nyctinomus* is found, namely *Cheiromeles*, which is restricted to the Indo-Malayan subregion, and *Molossus*, which is confined to tropical and subtropical America, excluding the United States. Notwithstanding the extended range of *Nyctinomus*, the species are closely related. With the exception of *N. johonensis* and *N. australis*, few specialized structures are met with; and but two species—one from Madagascar (*N. albiventer*) and a second from Africa (*N. acetubulosus*)—depart from a single formula for the teeth. As is the case with the *Cheiroptera* generally, the American species are the most obscure. Of the four described species, I have seen *N. brasiliensis*, *N. macrotis* and *N. gracilis*. A recent study of the materials at hand has led me to record descriptions of two new species.

**NYCTINOMUS EUROPS, n. s.**

Muzzle divided in middle into two parts by a vertical linear groove, the sides of which are defined by spines. These are continuous with the spines of the upper border of the muzzle. Ears united over the face for a